Polarization sensitivity in Collembola: an experimental study of polarotaxis in the water-surface-inhabiting springtail, *Podura aquatica*

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SUMMARY STATEMENT

In behavioural choice experiments we showed that the water springtail *Podura aquatica* possesses polarotaxis besides phototaxis, and we revealed fine details about its attraction to linearly polarized light.

ABSTRACT

The 6-ommatidium ventral eye of the water-surface-inhabiting springtail *Poduara aquatica* has horizontal and vertical microvilli and perceives light from the ventral, frontal and frontodorsal regions, while the 2-ommatidium dorsal eye possesses two upward-looking ommatidia with vertical microvilli. The ventral eye may serve water detection by its polarization sensitivity, even if the insect is resting with its head slightly tipped down on a raised surface. The polarization sensitivity and polarotaxis in springtails (Collembola) have not been investigated. Therefore, we performed behavioural choice experiments to study them in *P. aquatica*. We found that the strength of phototaxis in *P. aquatica* depends on the polarization characteristics of stimulating light. Horizontally and vertically polarized light were the most and least attractive, respectively, while unpolarized stimulus elicited moderate attraction. We showed that horizontally polarized light attracted more springtails than unpolarized, even if the polarized stimulus was 10 times dimmer.

36Thus, besides phototaxis, *P. aquatica* also has polarotaxis with the ability to measure or at least 37estimate the degree of polarization. Our results indicate that the threshold d^* of polarization 38sensitivity in *P. aquatica* is between 10.1 and 25.5 %.

39

40**Key words:** Collembola, Springtail, *Podura aquatica*, Polarization sensitivity, Polarotaxis,

41 Water detection, Visual ecology

42

43INTRODUCTION

44Springtails (Collembola) are abundant in all continents, even under the extreme conditions of 45Antarctica. The majority of the almost 7000 Collembola species is an important element of the 46terrestrial ecosystems. They live in the soil, feed on decaying plant matter and soil fungi (Rusek, 471998). However, some species, like *Podura aquatica* (Linnaeus, 1758) are the inhabitants of water 48surfaces (Shaller, 1972; Kriska, 2013). It has been shown that *P. aquatica* springtails strongly 49depend on water as they can be easily dehydrated through their thin cuticle by transpiration, and 50damages on the cuticle increase the transpiration rate. Restoring the speed of water loss to the 51normal level is achieved by regular moulting (Noble-Nesbitt, 1963a,b).

52 Generally, the cuticle of *P. aquatica* is unwettable, and the water surface acts as a membrane 53on which springtails can walk. Alive springtails submerged by water waves are surrounded by a thin 54silvery air layer, the buoyant force of which lifts them back to the water surface. On their first 55abdominal segment they have a hydrophilic ventral tubular appendage called the collophore, the 56main functions of which are excretion, water intake and adhesion to the water surface (Noble-57Nesbitt, 1963c; Hopkin, 1997). Collembola, especially water-inhabiting species like *P. aquatica*, 58also possess a forked, unique locomotory organ, the furcula attached to the fourth abdominal 59segment. The furcula is generally folded under the body, but when released, it snaps backwards and 60springs the animal upward providing a quick escape from predators (Hopkin, 1997; Kriska, 2013).

61 Aquatic insects detect water by means of the horizontal polarization of water-reflected light, 62and are guided to their water habitats by polarotaxis, that is attraction to this light characteristic 63(Schwind, 1983, 1984, 1989, 1991, 1995, 1999; Wildermuth, 1998; Horváth and Varjú 2004; Csabai 64*et al.*, 2006; Manor *et al.*, 2009; Horváth *et al.*, 2008; Egri *et al.*, 2012; Horváth, 2014). Until now, 65the polarization sensitivity and polarotaxis of Collembola have not been investigated.

66 Former studies have demonstrated that the photoreceptors in several springtail species also 67possess microvillar arrangement which may enable them to perceive light polarization (Paulus, 681972; Meyer-Rochow *et al.*, 2005). The phototactic behaviour of various Collembola species has 69been studied, and the results showed negative phototaxis except for species living on water surfaces 70or plants (Shaller, 1972; Salmon and Ponge, 1998; Dromph, 2003; Fox *et al.*, 2007), such as *P.*

71*aquatica*. The ecological reason for negative phototaxis in the majority of springtails is that they
72live in the soil and light indicates inappropriate habitat which should be avoided. Besides
73phototaxis, geotaxis (Boiteau and MacKinley, 2014) and shape perception (Shaller, 1972) have also
74been demonstrated in Collembola.

75 The number of ommatidia in the eyes of springtails varies within species from a maximum
76of eight to the total absence. *Podura aquatica* has 8 ommatidia in a "double eye" partitioned to a
77dorsal and a ventral eye region, and the orientation of each ommatidium is also known (Paulus,
781970). The ventral and dorsal eye regions are composed of six and two ommatidia, respectively.
79The ventral eye region is equipped with strictly horizontal and vertical (orthogonal) microvilli and
80perceives the light from the (i) ventral, (ii) frontal and (iii) frontodorsal regions, while the two
81upward-looking dorsal ommatidia possess only vertical microvilli (Fig. 1A). Due to the wide (up to
8280°) opening angles of the collembolan ommatidia (Shaller, 1972), the field of view of the ventral
83eye region is presumably not limited to the lower hemisphere relative to the head, however the
84exact opening angles of the ommatidia in *P. aquatica* has not been studied yet. Hence, it is
85presumable that the ventral eye region may also serve water detection by its polarization sensitivity,
86even if the animal is resting with its head slightly tipped down on a raised surface (Fig. 1B).

87 From the fact that orthogonally aligned microvilli are present in the ventral eye region of the
88water springtail *P. aquatica*, it does not follow that this species possesses polarotaxis, although this
89is a reasonable hypothesis because of its strong dependence on water. Therefore, we performed
90behavioural choice experiments to study the polarization sensitivity and polarotaxis in this
91collembolan species.

92

93**MATERIALS AND METHODS**

94**Springtails**

95*Podura aquatica* adults (males and females) were collected from the surface of ponds and creeks in
96the vicinity of Budapest, between March and June 2015. The springtails were kept in laboratory at
9710 °C under 12:12 dark:light conditions in jars containing original pond-water and aquatic plant
98leaves.

99

100**Choice-box**

101The primary equipment of our experiments was a choice-box possessing two windows for light
102stimuli with variable polarization characteristics (Fig. 2). The arena was composed of a small
103aquarium (30 cm × 20 cm × 10 cm), the inner and outer surface of which was covered with matte
104white paper except for two square (5.6 cm × 5.6 cm) areas on the two ends of the aquarium making
105up windows for the light stimuli (Fig. 2A,B). The matte white paper ensured the minimization of

specular reflections and unwanted polarization signals. The choice-box had a removable cover with a circular hole through which the interior of the arena could be recorded by a digital camera (Fig. 2A). The inner surface of the cover was also matte white, thus the tested springtails moving at the arena bottom saw a homogeneous matte white environment except for the two stimulus-windows and the objective lens of the camera at the center of the top element (Fig. 2B). On the bottom of the choice-box an exchangeable matte white sheet of paper was placed with two printed black lines dividing the box into three equal partitions and a printed black circle at the center of the paper representing the release location of springtails.

Depolarizer array

The polarization characteristics of each light stimulus were variable discretely by means of a linearly polarizing sheet (XP42-18, ITOS, Mainz, Germany) housed in a rigid cardboard frame and a series of 15 slightly depolarizing sand-blasted glass panes between two ordinary, colourless, transparent, non-polarizing and non-depolarizing glass layers fixed in a wooden U-profile (Fig. 2C). Therefore, 3 mm wide gaps between the neighbouring glass layers formed 16 slots, where the frame with the polarizer could be inserted. The white unpolarized light emitted by a Ledion LB-P38-153100 cool LED lamp (640 lumens) entered the U-profile and penetrated through all of the glass layers and the polarizer. The transmission axis of the polarizer in the frame and the number of slot the frame was slipped into (S_i, i = 1..16) determined the angle α and degree *d* (%) of polarization of the stimulus: The closer the polarizer was to the LED lamp, the lower the *d* of light stimulus was, because the light must have passed more depolarizing glass layers after leaving the polarizer. Two layers of matte white common office paper were also inserted into slot S_{15} (2nd slot from the LED lamp) to ensure the total depolarization of light (Fig. 2C). Consequently, inserting the polarizer into slot S_1 (furthest from the LED lamp) or S_{16} (closest to the LED lamp) created 100% linearly polarized or practically unpolarized light stimulus, respectively. Finally, a wooden lid covered the depolarizer array at the top. We prepared 10 frames holding a polarizer sheet, each with differently orientated transmission axis, thus the angle of polarization α could be varied with a 10° step between the horizontal (α = 0°) and vertical (α = 90°) by inserting the proper polarizer-holding frame into the desired slot of the depolarizer array.

The degree of polarization *d* of the stimulus was measured as a function of the polarizer position in the red (650 nm ± 50 nm), green (550 nm ± 50 nm) and blue (450 nm ± 50 nm) parts of the spectrum with a NIKON D3200 digital camera equipped with a calibrated polarizer (W-Tianya Slim MC CPL). Shooting images with three polarizer angles in RAW format (the linear voltage response of the CMOS pixels as a function of light intensity as recorded in the RAW image was verified by the Estrato Research & Development Ltd., www.estrato.hu) enables the experimenter to

141calculate the degree d and angle α of polarization pixel by pixel (Horváth and Varjú, 1997, 2004). At
142each slot setting the d-values obtained at the pixels corresponding to the stimulus window were
143averaged and the results of the three spectral bands were also averaged. Figure 3 shows the d- and
144α-patterns of an unpolarized (Fig. 3A-C), 100% horizontally polarized (Fig. 3D-F) and 100%
145vertically polarized stimulus (Fig. 3G-I) with the choice-box interior in the green (550 nm) spectral
146range.

147 Independently of the polarizer position, the spectral characteristics of the light stimuli were
148the same, since the same materials of the same number occupied the optical path. The emission
149spectrum of the light stimuli, which was measured with an Ocean Optics STS-VIS spectrometer in
150the visible spectral range, had a major and a minor peak at 550 and 450 nm, respectively (Fig. 2D).

151 On the other hand, the intensity of the light stimulus had a slight dependence on the
152polarizer position, because the frame of the insertable polarizer did not block the whole cross-
153section of the depolarizer array near the immediate bottom of the lid. We measured this dependence
154for horizontally and vertically polarized stimuli by taking photographs from the other stimulus
155window in RAW format with the same camera settings, and finally summing all pixel values for
156each image. Then we normalized the total intensity values with the 100% polarized case which was
157the maximal value. As Fig. 4A. shows, we obtained a monotonic increase in intensity from the
158unpolarized to the 100% polarized case and the ratio of the two extremes was $I_{unpol}/I_{pol} = 0.84$. The
159difference between the vertically and horizontally polarized calibration curves was negligible.

160 To test how the matt white coating affects light reflection as a function of polarization, we
161also measured and compared the total wall-reflected intensities with the direct stimulus intensities
162in case of 100% horizontally and 100% vertically polarized stimulus as a function of the degree of
163polarization (polarizer position). Figure 4C shows a RAW image with the stimulus window and the
164interior of the choice-box. The ratio of the summed pixel values of the reflections (outside the red
165rectangle) and the direct stimulus (inside the red rectangle) was calculated for each image. Dividing
166with the maximum value resulted in the normalized I_{refl}/I_{stim} ratio as a function of the degree of
167polarization (Fig. 4B). It is clear that the intensity ratio of the reflections and the direct stimulus was
168significantly less than 4% in case of the majority of the polarizer positions. In other words, the
169Weber contrast between the reflection-related disturbances and the direct stimulus was significantly
170low.

171

172**Test trials**

173The tests with *P. aquatica* were performed in choice trials, the concept of which was the following:
174At first, to minimize the influence of odours, a new matte white paper sheet with the black
175partitioning lines was placed onto the bottom of the choice-box, and an opaque plastic releaser tube

176(diameter = 28 mm, height = 14 cm) was stood at the box center. 100-250 *P. aquatica* specimens 177were placed into the releaser, the cover of the choice-box was set up, the desired light stimuli were 178applied (Fig. 2C-D), and the laboratory was darkened. After 30 seconds, the releaser was removed 179and 10 photographs (6016 × 4000 pixel resolution, JPEG format) were taken encompassing 81 180seconds. Then, the cover was detached and the springtails were collected from the choice-box. In 181order to eliminate artefacts arising from the incidental slight differences in the LED light sources 182and the two sides of the arena, each trial was repeated with reversed stimulus arrangement. Thus, 183we measured Collembola reactions to different stimulus pairs in even number of trials and equal 184numbers of trials were carried out for each stimulus configuration. Furthermore, to avoid pseudo-185replication, always new specimens were introduced in each trial. In this way, altogether 25407 *P.* 186*aquatica* specimens were tested in 5 experiments covering 164 trials (Table 1). An additional 300 187springtails were also tested individually in experiment 6 as described later. The relative humidity 188was measured with a HIH-4000 Series humidity sensor in the laboratory and varied between 45 and 18950% during the experiments.

190

191**Evaluation and statistics**

192In the 10 photographs taken during every trial (Fig. 5A-C), the position of each springtail (being the 193only non-static objects in the arena) was determined by a custom-developed software written in 194GNU OCTAVE 4.0 (Fig. 5, for details of the algorithm and the software please contact the 195corresponding author). For each trial, as the first step, the static background image of the choice-196box was obtained by calculating the median of the 10 images (Fig. 5E). Subtracting the inverse of 197the background image from the inverse of a given photograph resulted in a new image containing 198only the springtails as bright patches on a black background (Fig. 5F). This image was thresholded 199with the method of Otsu (1979), and the number and centroid position of the patches were 200determined (Fig. 5G). The two black lines on the underlying white paper perpendicular to the longer 201edge of the choice-box were also recognized by the software, thus it could be determined 202automatically if a given springtail was located in the left, middle or right third of the choice-box 203(Fig. 5I). To minimize errors, the detection of springtails were checked manually in case of all 204photographs, and the threshold level was adjusted if it was necessary.

205 In the first photograph (t = 0 s), the springtails were crowded at their starting position (black 206circle). Later, they dispersed and shortly several specimens approached the wall of the choice-box, 207and a few got under the replaceable paper sheet. Since the automatic detection underestimated the 208number of springtails when they were initially crowded at a relatively small area, the total number 209of specimens was determined correctly later, when they dispersed, but still did not have time to get 210under the paper. Thus, for each trial, the maximal number of detections from the 10 images was

211considered as the number of springtails participating in the given trial. Figure 5H shows the mean
212number of detected springtails as a function of the image number for all 164 trials. The maximal
213value occurred at file number 6 (t = 45 s), thus the chosen 81 s long duration for the trials was
214justified.

215 In order to quantify the reaction strength of the several hundred *P. aquatica* at a given
216stimulus setting, we calculated the mean position-shift of springtails toward one of the sides (e.g.
217polarized stimulus) relative to the choice-box center for the last photograph (t = 81 s) corresponding
218to the given stimulus pair. For example, in experiment 4, we tested the preference of springtails to
219polarized light against unpolarized one in four trials in each stimulus configuration. Thus, for a
220given stimulus pair we calculated the centroid of springtail positions toward the polarized stimulus
221including all four photographs taken at t = 81 s (in case of swapped stimulus settings the horizontal
222coordinates were multiplied by –1). We defined the relative centroid shift Δx as

223

224 $$\Delta x = x / L, \quad (1)$$

225

226where x is the horizontal coordinate of the centroid of springtail positions, and L is the length of the
227choice-box, both measured in pixels. The other quantification method we used for determining the
228significance of reactions in a given stimulus setting was to compare the number of springtails in the
229two terminal thirds of the arena at the end of the trial (last photograph, t = 81 s) with χ^2 test. The
230specimens in the middle third were treated as inactive and were ignored, even though they were
231moving. Presuming a linear relationship between light intensity and the strength of phototaxis, for
232the χ^2 tests, in case of experiments 4, 5 and 6, we modified the expected number of responses
233linearly proportionally to the intensities of the two stimuli (Fig. 4A) in order to compensate for the
234slight intensity differences. For example, in experiment 6 when 100 springtails were tested and the
235stimuli were unpolarized and 100% horizontally polarized, the expected number of responses were
236modified to 45.652 and 54.348 based on the I_{unpol}/I_{pol} = 0.84 intensity ratio (Fig. 4A).

237

238**Experiment 1: Control**

239In order to test the homogeneity of the choice-box, we performed control trials in which both
240optical stimuli were unpolarized with equal intensity: On both sides of the choice-box the polarizer
241was inserted into slot S_{16} of the depolarizer array to produce unpolarized stimulus (with degree of
242polarization $d \approx 0\%$).

243

244**Experiment 2: Phototaxis**

245In this experiment we tested the phototactic reactions of *P. aquatica* in three cases: At one side of

246the choice-box, the LED light source was turned off (dim stimulus), and the other stimulus was (i)
247100% horizontally polarized light, (ii) 100% vertically polarized light, and (iii) unpolarized light
248with operating LED light (polarizer inserted into slot S_1).
249

250**Experiment 3: Polarotaxis *versus* phototaxis**

251Here we tested the preference of *P. aquatica* to 100% horizontally polarized light against
252unpolarized light with dimmer light intensities on the polarized side of the arena: The intensity ratio
253I_{pol}/I_{unpol} of the polarized and unpolarized stimulus varied between 0.063 and 1.140. The intensity of
254the polarized stimulus was changed by inserting an additional frame containing a polarizer sheet
255with different oblique transmission axes into slot S_2, next to the horizontal polarizer placed in slot
256S_1. According to the Malus law, the transmitted intensity of 100% polarized light through a linear
257polarizer is proportional to $\cos^2\beta$, where β is the angle between the direction of polarization of
258incoming light and the transmission axis of the polarizer. In this way, the transmission axis of the
259polarizer in slot S_2 determined the intensity of light stimulus exiting the horizontal polarizer in slot
260S_1. The exact intensities were measured with the same digital camera by extracting the pixel
261information of the stimulus window from RAW images. The outcome of this experiment revealed
262whether *P. aquatica* possesses polarization vision, or if only the strength of the horizontally
263polarized component of the stimulus influences its reaction.
264

265**Experiment 4: Varying degree of polarization *d***

266In this experiment we tested the preference of *P. aquatica* to horizontally and vertically polarized
267light against unpolarized one as a function of the degree of polarization *d*. Different *d*-values were
268produced by using slots S_1, S_2, S_3, S_4, S_5, S_6, S_8, S_{10}, S_{14} and S_{16} of the depolarizer arrays. The
269resulting *d*-values (averaged over the visual spectral range) were 100.0, 95.9, 87.4, 77.2, 66.5, 55.9,
27038.1, 25.5, 10.1 and 2.6%, respectively. These values are the averages of pixel-by-pixel
271measurements in three spectral bands (R, G, B) as described above. The standard deviation was less
272than 2.5% in all cases. The ratio of the intensities of the unpolarized and polarized stimuli is shown
273in Fig. 4A as a function of *d*.
274

275**Experiment 5: Varying angle of polarization α**

276In this experiment we tested the reaction of *P. aquatica* to varying angle of polarization α of 100%
277polarized light against an unpolarized stimulus. The ratio of the intensities of the polarized and
278unpolarized stimuli was $I_{unpol}/I_{pol} = 0.84$ (Fig. 4A). α was changed between the horizontal and
279vertical with 10° steps.
280

281**Experiment 6: Tests with individual springtails**

282As numerous springtails were involved simultaneously in each trial, the question arises whether the 283behaviour of a given springtail might have been affected by others. The ideal method would be to 284test each springtail separately, independently of the others. However, this technique would be very 285time-consuming due to the thousands of specimens. To show that the reactions were not appreciably 286affected by the presence of other specimens in the choice-box, we performed experiment 6, in 287which we introduced the springtails one-by-one. We tested three situations each with 100 288springtails: (i) 100% horizontally polarized *versus* unpolarized light, (ii) 100% vertically polarized 289*versus* unpolarized light, and (iii) unpolarized *versus* unpolarized stimulus as a control experiment. 290The ratio of the intensities of the polarized and unpolarized stimuli was $I_{unpol}/I_{pol} = 0.84$, and was 291$I_{unpol}/I_{pol} = 1$ in the third case. After release, at the 81st second, the position (left, right or middle 292partition) of the single springtail was registered visually through the circular hole on the cover. The 293stimulus arrangement was swapped after every fifth test.

294

295**Additional methodological and analytical information**

296(i) *Podura aquatica* is not protected species in Hungary, therefore no permission was necessary for 297our experiments. (ii) No human subjects were involved in our work. (iii) No reagents were used.

298

299**RESULTS**

300The results of our experiments provided detailed information about the polarization sensitivity as 301well as polarotactic and phototactic behaviour of *P. aquatica* in the visible spectral range. Table 1 302shows the numbers of trials and tested *P. aquatica* in our six experiments. Table 2 contains the 303measured relative centroid shift Δx of springtail positions in experiments 1 and 2 with the statistical 304significance of reactions.

305 In experiment 1 we tested the homogeneity of the choice-box in control trials. It is clear 306from Table 2 that the value of Δx was practically zero, and left-right reactions of springtails showed 307no significant difference ($\chi^2 = 0.54$, df = 1, p = 0.4624). Hence, the attractiveness of both identical 308unpolarized stimuli was the same to Collembola.

309 The results of experiment 2 show unambiguous positive phototaxis in *P. aquatica*. However, 310the reaction strength depended on the polarization characteristics of the light stimulus. According to 311Table 2, springtails preferred the bright side of the choice-box against the dim side. The relative 312centroid shift Δx toward the polarized stimulus was 0.0847, 0.0576 and 0.0186 when the light 313stimulus was 100% horizontally polarized, unpolarized and 100% vertically polarized, respectively. 314According to the χ^2 tests, the reactions were significant, except for the last one (Table 2).

315 Figure 6 shows the reactions of springtails as a function of the intensity ratio I_{pol}/I_{unpol} of the

316polarized and unpolarized stimulus in experiment 3, where the phototaxis was compared with
317polarotaxis. The exact number of choices at the terminal thirds and the relative centroid shift Δx
318toward the 100% horizontally polarized stimulus are shown in Fig. 6A and 6B, respectively. The
319springtails were most attracted to the polarized stimulus when the intensity ratio of the polarized
320and unpolarized stimulus was maximal ($I_{pol}/I_{unpol} = 1.14$). As the intensity of the polarized stimulus
321decreased, its attractiveness dropped also and became zero when the polarized stimulus was more
322than 10 times dimmer than the unpolarized one. At intensity ratio $I_{pol}/I_{unpol} = 0.063$ the phototaxis
323overwhelmed the polarotaxis, and the springtails preferred the unpolarized stimulus.

324 In experiment 4 we studied the responses of springtails to horizontally and vertically
325polarized light as a function of the degree of polarization d against unpolarized stimulus. Figure 7A
326shows the number of choices at the terminal thirds of the choice-box corresponding to the polarized
327and unpolarized stimuli, while Fig. 7B displays the relative centroid shift Δx of springtail positions
328as a function of d. The black and grey bars correspond to the horizontally and vertically polarized
329stimulus (Fig. 7A,B) and the white ones to the unpolarized stimulus (Fig. 7A). In general, *P.*
330*aquatica* preferred the horizontally polarized light against the unpolarized one, while in the
331presence of vertically polarized and unpolarized stimuli they preferred the unpolarized light. The
332reaction strength of springtails increased with increasing d.

333 The reactions of springtails, when the angle of polarization preference was tested against the
334unpolarized stimulus in experiment 5, are shown in Fig. 8. Figure 8A displays the number of
335choices at the terminal thirds corresponding to the 100% polarized and unpolarized stimuli, and Fig.
3368B shows the relative centroid shift Δx of springtail positions toward the polarized stimulus as a
337function of the angle of polarization α of the 100% polarized stimulus. Springtails were most
338attracted to the horizontally polarized light ($\alpha = 0°$) and moved away from the vertically polarized
339stimulus ($\alpha = 90°$). In the case of intermediate α-values, a transition occurred around $\alpha = 50°$ where
340the distribution of springtails showed no preference for any stimulus.

341 Table 2 shows the reactions of individual *P. aquatica* springtails tested in experiment 6.
342Springtails preferred the 100% horizontally polarized light against the unpolarized one ($N_{pol} = 47$,
343$N_{unpol} = 7$, $N_{inactive} = 46$). At the same time, they were attracted to the unpolarized stimulus when the
344other was 100% vertically polarized ($N_{pol} = 9$, $N_{unpol} = 47$, $N_{inactive} = 44$). In both cases the differences
345were highly significant. There was no significant difference when both stimuli were unpolarized
346($N_{left} = 28$, $N_{right} = 23$, $N_{inactive} = 49$).

347

348**DISCUSSION**

349Before drawing conclusions from our results, it is important to make sure of the symmetry of the
350used choice-box. The suitability of our choice-box was verified by the outcome of experiment 1,

351showing no significant spatial bias in the springtail distribution between two optically equivalent,
352unpolarized stimuli (first row of Table 2).

353 In addition to verifying the positive phototactic behaviour of *P. aquatica* springtails (Shaller,
3541972), in experiment 2 we showed that the strength of their attraction to light depends on the
355polarization characteristics: According to Table 2, the attraction was the strongest and the weakest
356when the bright stimulus was 100% horizontally and vertically polarized, respectively. The
357unpolarized stimulus elicited an intermediate, moderate attraction from springtails. Although the
358intensity ratio of the unpolarized and any kind of 100% polarized stimulus was $I_{unpol}/I_{pol} = 0.84$, the
359comparison of the attraction to 100% horizontally and 100% vertically polarized light raises the
360reasonable suspicion that phototaxis and polarotaxis coexist in *P. aquatica*.

361 If only the horizontally polarized component of the light stimulus had played a role in the
362attraction of springtails, their distribution would have been symmetrical in the case of an intensity
363ratio $I_{pol}/I_{unpol} = 1/2$ in experiment 3, because the horizontally polarized component of an unpolarized
364stimulus has half the intensity of the unpolarized stimulus itself. As shown in Fig. 6, the relative
365centroid shift Δx of springtail positions toward horizontally polarized light was positive, even if the
366polarized stimulus was 10 times dimmer than the unpolarized one. For each tested intensity ratio,
367the significances of the χ^2 tests are shown by asterisks in Figure 6A. This fact obviously confirms
368the assumption, that besides phototaxis, polarotaxis is also present in *P. aquatica* with the ability to
369measure or at least estimate the degree of polarization d of stimulating light. Similar coexistence of
370phototaxis and polarotaxis has been shown in numerous aquatic beetles, furthermore a synergistic
371interaction between both taxa has also been demonstrated (Boda *et al.*, 2014).

372 In experiments 4 and 5 more details about the nature of polarotaxis of *P. aquatica* could be
373revealed. For polarotactic aquatic insects the degree of polarization d of water-reflected light is also
374a crucial parameter. As shown in Fig. 7, in experiment 4 the springtails did not express any
375significant reaction if the polarizer was inserted into slot S_{14}. From this we conclude that the
376threshold d^* of polarization sensitivity in *P. aquatica* is between 25.5% (slot S_{10}) and 10.1% (slot
377S_{14}). The threshold of polarization sensitivity of the dorsal rim area in terrestrial field crickets
378(Labhart, 1996) and honey bees (von Frisch, 1967; Rossel and Wehner, 1984) is $d^* \approx 5\%$ and $d^* \approx$
37911%, respectively. In behavioural field tests, Kriska *et al.* (2009) measured d^* in polarotactic
380dragonflies ($d^* \approx$ 0-24%), mayflies ($d^* \approx$ 32-92%) and tabanid flies ($d^* \approx$ 32-92%). Hence, in *P.*
381*aquatica* the values of d^* that can elicit positive polarotaxis are similar to that of dragonflies. The
382degree of polarization of water-reflected light is maximal at the Brewster angle, when the reflected
383light beam is perpendicular to the refracted one ($\theta_{Brewster} \approx 53°$ for the water surface measured from
384the vertical). According to Gál *et al.* (2001), Bernáth *et al.* (2004) and Horváth (2014), the degree of
385polarization reflected by dark waters from the Brewster angle can reach $d \approx 80\%$, practically

386independently of the solar elevation and sky conditions (clear or cloudy). For bright waters, the
387maximum of d can drop to about 25%, thus it can be questionable whether these waters can be
388detected polarotactically by aquatic insects with polarization sensitivity thresholds higher than 25%.
389According to the relatively low threshold of polarization sensitivity in *P. aquatica* (10.1% < d^* <
39025.5%), we conclude that the water springtail is equipped with a highly water-sensitive sensory
391system.

392 Based on our results, the attractiveness of various directions of polarization of light is the
393following: The most attractive stimulus was 100% and horizontally polarized, the unpolarized light
394elicited moderate attraction, and the least attractive was the 100% vertically polarized stimulus
395(Table 2).

396 In experiment 5, compared to unpolarized light, springtails were attracted to horizontal
397polarization and avoided vertical polarization (Fig. 8). The transition angle α^* (from the horizontal)
398at which springtails equally preferred the 100% polarized and unpolarized ($d \approx 0\%$) stimulus, was
399not 45°, but closer to 50°. This slightly asymmetric reaction in experiment 5 possibly arises from
400the slight intensity differences between the 100% polarized and unpolarized stimuli. Similar
401asymmetry occured in experiment 4 where various degrees of polarization were tested against
402unpolarized stimulus: The attraction to horizontally polarized light was stronger than the avoidance
403of vertically polarized light. The reason may have been the slight intensity difference between the
404polarized and unpolarized stimuli, but for the exact answer an additional experiment should be
405performed with equal stimulus intensities.

406 In experiment 6 we demonstrated that testing many (100-250) Collembola specimens
407simultaneously was a sound method, because the springtails tested individually expressed the same
408reactions (Table 2) as their counterparts in simultaneous experiments conducted with multiple
409springtails (experiment 4: horizontal polarizer in S_1, vertical polarizer in S_1, polarizer in S_{16}, Fig. 7).

410 Since *P. aquatica* springtails have horizontal and vertical microvilli in their ventral eye
411region (Paulus, 1972) and in our present study they showed unambiguous polarotaxis, we suppose
412that this species possesses a visual system that enables it to detect water by means of the horizontal
413polarization of water-refleced light, as is the case in many other polarotactic aquatic insect species
414(reviewed in Horváth, 2014). Labhart (1988) demonstrated the presence of polarization opponent
415neurons which connect photoreceptors with orthogonal microvilli in crickets. We hypothesize a
416similar mechanism in *P. aquatica* where the sensed contrast between the horizontal and vertical
417microvillar systems offers the ability to estimate the angle and degree of polarization of light: 100%
418horizontally polarized, unpolarized and 100% vertically polarized light are points along a contrast
419gradient which determines the attractiveness. Our results highly support this concept, especially
420experiment 3. The outcomes of experiments 2, 4, 5 and 6 do not really require the springtails to

421estimate or measure the degree of polarization. If *P. aquatica* was just phototactic that detects only
422horizontal polarization (possesses only one, horizontal microvilli arrangement in all ommatidia), the
423latter experiments could give similar results. At the same time, the distribution of springtails in
424experiment 3 would have been expectedly symmetrical when the intensity ratio of the 100%
425polarized and unpolarized stimuli was $I_{pol}/I_{unpol} = 1/2$. In reality, the springtails preferred the 100%
426horizontally polarized light against unpolarized light even if the intensity of the former was 10
427times dimmer. Consequently, *P. aquatica* has the ability to estimate the degree of polarization.
428Obviously, our findings are valid only in the visible spectral range, since our setup was not able to
429produce ultraviolet light. The spectral sensitivity of *P. aquatica* has not been measured yet, but
430expectedly the sensitivity has at least one peak in the visible spectral range.

431 Since the few (2 in the dorsal eye region and 6 in the ventral one) ommatidia of *P. aquatica*
432possess relatively large opening angles (Shaller, 1972), the field of view of the ventral eye region is
433capable of detecting water surfaces, even if the insect is crawling on a raised surface with its head
434tipped down (Fig. 1). This anatomical feature allowed us to use light stimuli coming from above the
435horizon viewed by the tested springtails placed onto the bottom of the choice-box. The attraction to
436horizontally polarized light definitely serves the water detection and basically helps the springtails
437to stay in the immediate vicinity of water, since *P. aquatica* springtails usually do not leave their
438habitat. However, after dispersion by wind, springtails may utilize their polarization sensitivity in
439habitat seeking.

440 Unlike the ventral eye region, the upper two ommatidia composing the dorsal eye region
441have only vertical microvilli, and it is still to be studied whether the dorsal eye region of *P. aquatica*
442can or cannot exploit polarization information. It has been shown that *P. aquatica* and other
443Collembola species are able to orient and maintain a certain direction under natural and artificial
444radiance distributions (Verheijen and Brouwer, 1971; Hågvar, 2000; Manica *et al.*, 2000). However,
445it has not been studied whether springtail navigation and orientation are governed also by skylight
446polarization.

447

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456

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461

462**COMPETING INTERESTS**

463The authors declare no competing or financial interests.

464

465**AUTHOR CONTRIBUTIONS**

466Á.E., G.H., G.K. designed the experiments, Á.E., A.F. performed the experiments, Á.E. did the
467programming and analyzed the data. Á.E., A.F., G.H., G.K. wrote the paper and answered the
468comments of the Reviewers.

469

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564

565 # Tables

566

567**Table 1**: Number of tested *P. aquatica* springtails and number of trials in the six laboratory choice
568experiments.

569

experiment	number of springtails	number of trials
1	1727	10
2	3342	24
3	2470	18
4	10334	72
5	7534	40
sum of experiments 1-5	**25407**	**164**
6	300	300

570

571**Table 2:** Numbers *N* of individual springtails observed in the two terminal third of the choice-box 572in experiments 1, 2 and 6 with the statistical significance of reactions. Asterisks indicate if the 573expected values were modified based on the slight intensity differences in the stimuli. The relative 574centroid shifts Δx of springtail positions for experiments 1 and 2 are also given in the table.
575

exp.	stimulus	Choices		Δx	χ^2	df	p	significance
1	unpolarized *versus* unpolarized	N_{left}=259	N_{right}=276	0.0009 (toward right stimulus)	0.54	1	0.4624	no
2	100% horizontally polarized *versus* dim	N_{pol}=311	N_{dim}=118	0.0847 (toward polarized light)	86.83	1	< 0.0001	yes
	unpolarized *versus* dimmer unpolarized	N_{unpol}=522	N_{dim}=283	0.0576 (toward brighter light)	70.96	1	< 0.0001	yes
	100% vertically polarized *versus* dim	N_{pol}=188	N_{dim}=165	0.0186 (toward polarized light)	1.50	1	0.2209	no
6	100% horizontally polarized *versus* unpolarized	N_{pol}=47	N_{unpol}=7	-	37.23*	1	< 0.0001	yes
	100% vertically polarized *versus* unpolarized	N_{pol}=9	N_{unpol}=47	-	33.02*	1	< 0.0001	yes
	unpolarized *versus* unpolarized	N_{left}=28	N_{right}=23	-	0.49	1	0.4838	no

576

Figures with Legends

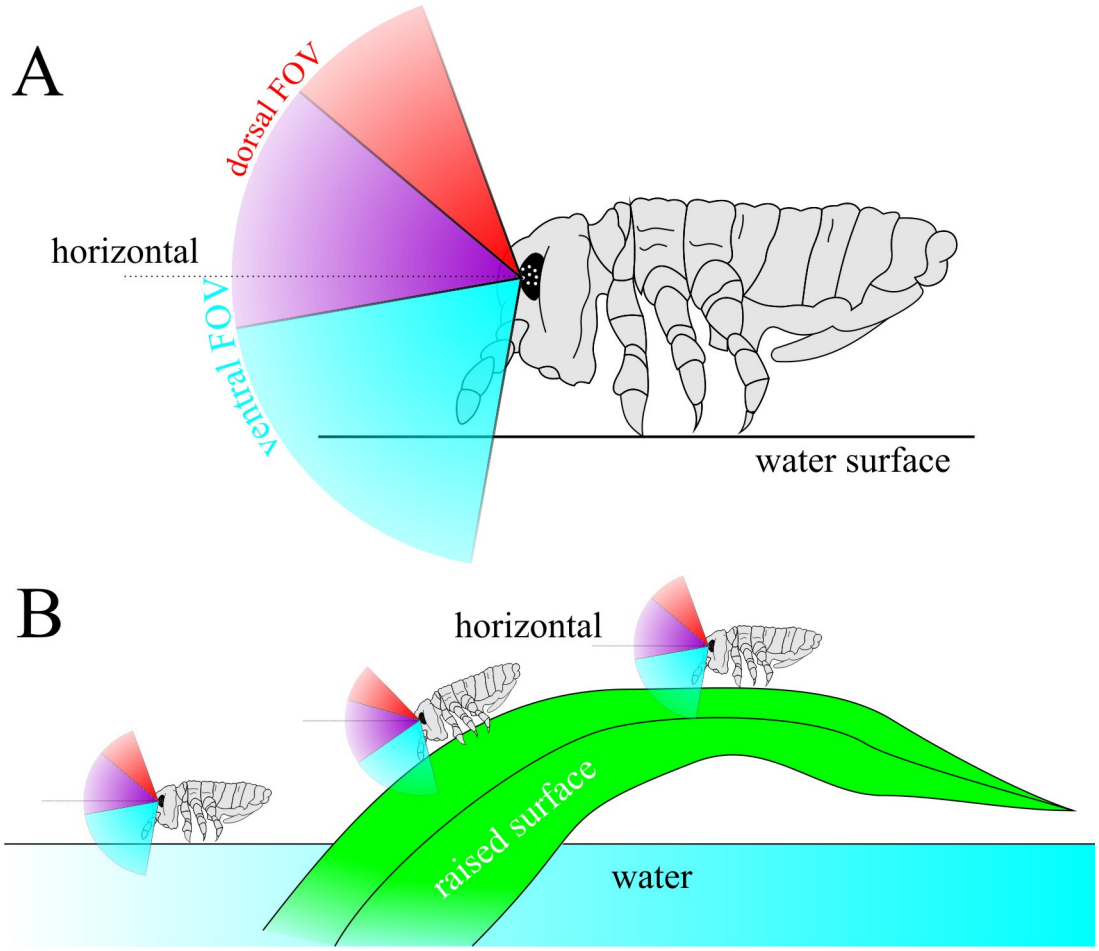
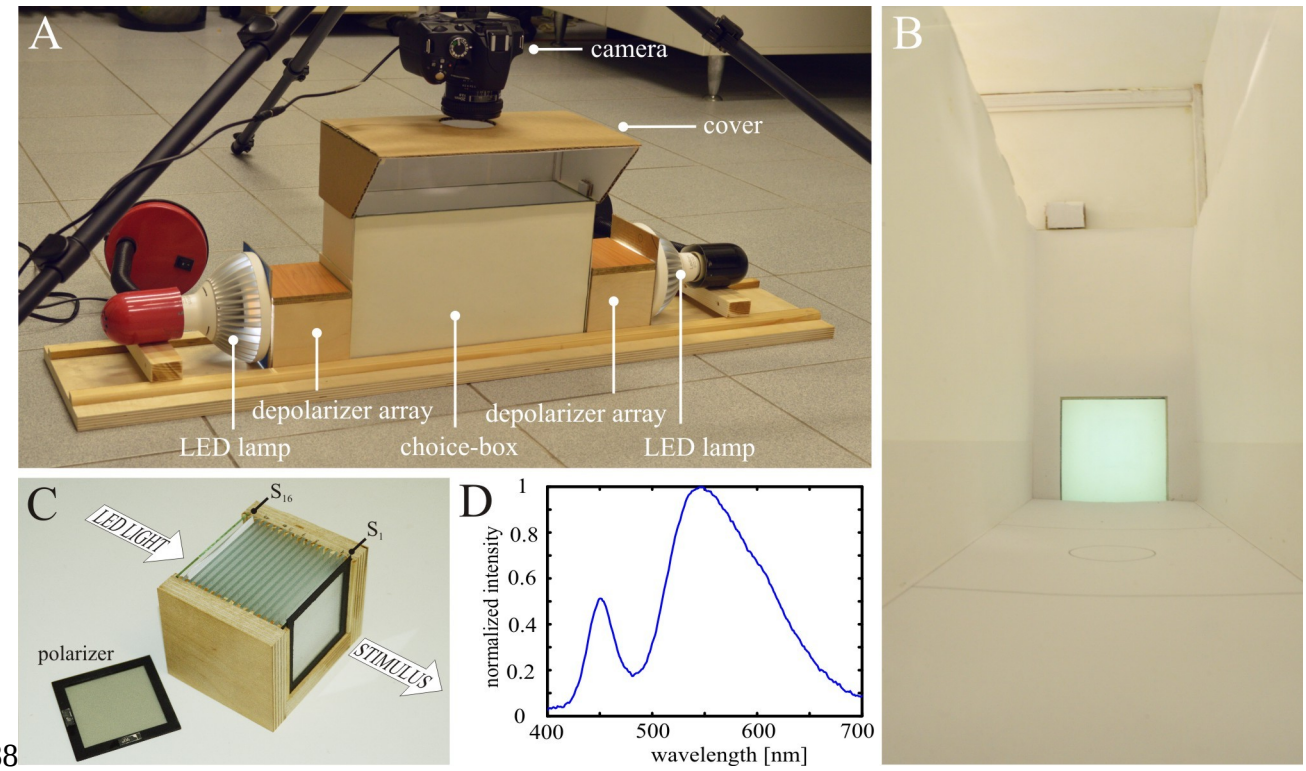


Figure 1: The field of view (FOV) of the "double eye" in *Podura aquatica*. (A) The slightly overlapping (violet) red and blue sectors represent the estimated field of view of the dorsal and ventral eye regions, respectively. (B) Demonstration of the role of the ventral eye region in water detection, even if the head is tipped down. The green leaf represents an arbitrary raised surface (e.g. soil, vegetation, gravel).



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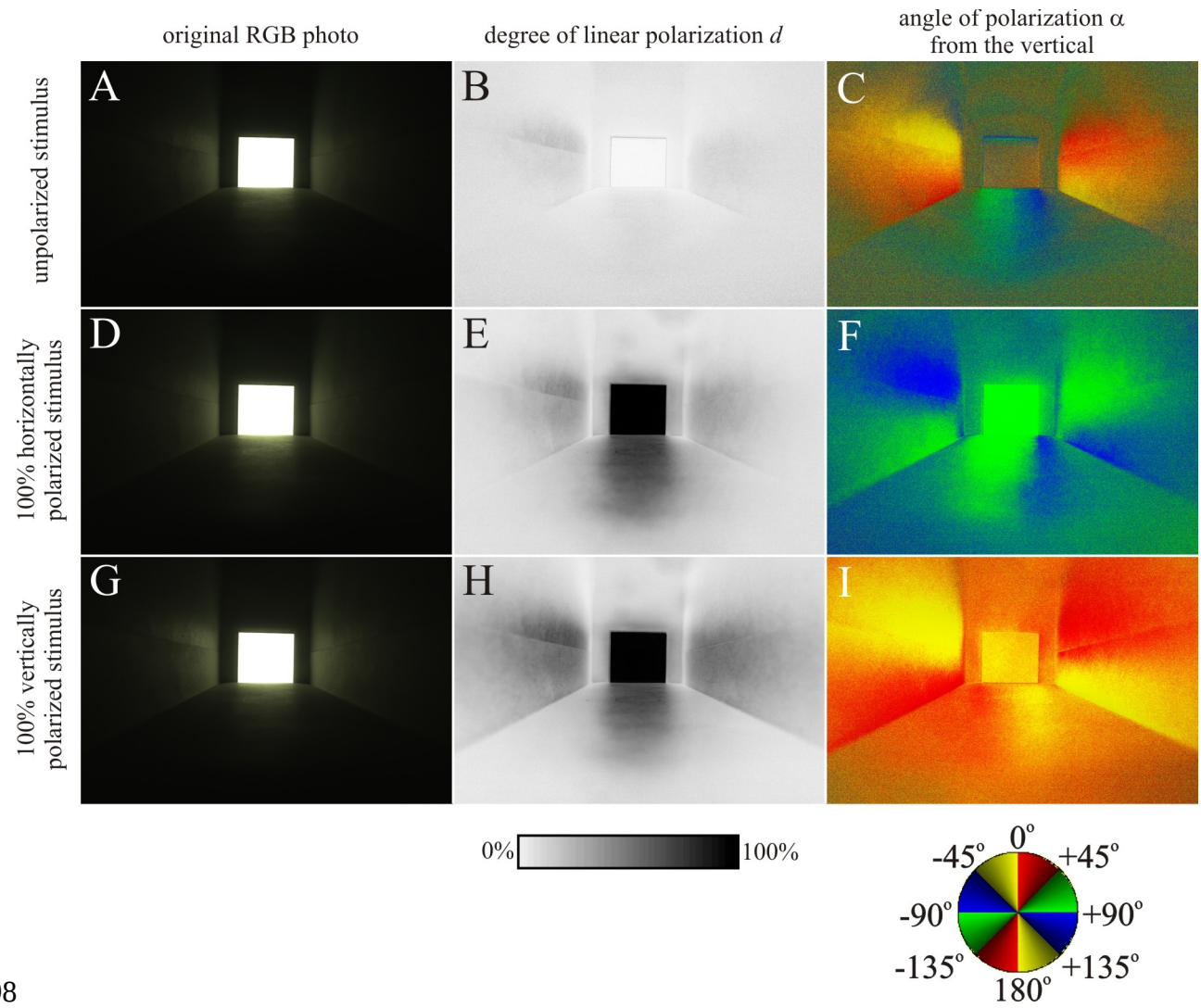
591**Figure 2: Overview of the experimental setup used for the choice experiments.** (A) Photograph
592of the setup. (B) Perspective from the point of view of a springtail from one end of the choice-box.
593The laboratory lights were turned off during the experiments. (C) An insertable linear polarizer in a
594rigid frame and the structure of the depolarizer array composed of two ordinary and 15 sand-blasted
595glass panes in a U-profile. In slot S_{15} (next to S_{16}) two layers of matte white office paper are
596inserted. (D) Emission spectrum of the light stimulus entering the choice-box.
597



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599**Figure 3: Imaging polarimetry of the interior of the choice-box with unpolarized (A-C), 100%** 600**horizontally polarized (D-F), and 100% vertically polarized (G-I) stimulus in the green (550** 601**nm) spectral range.** (A, D, G) Original RGB photographs. (B, E, H) Patterns of degree of linear 602polarization *d*. (C, F, I) Patterns of angle of polarization *α* measured clockwise from the vertical.
603

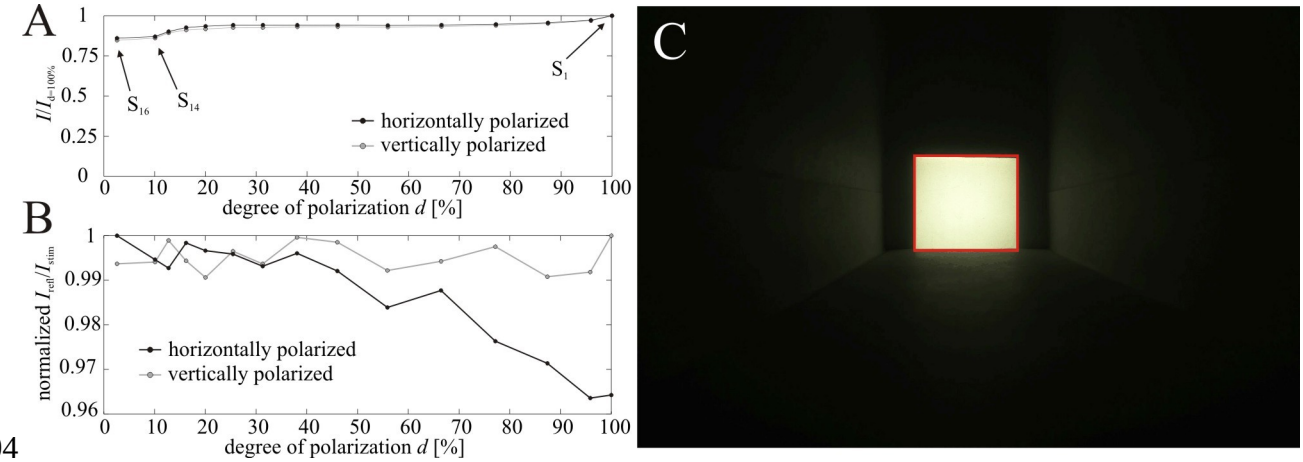


Figure 4: Intensity of the stimulus and the reflections on the walls as a function of the degree of polarization *d*. (A) Normalized intensity of light stimulus and reflections together as a function of d for horizontally and vertically polarized light. (B) Normalized ratio of the intensity of reflections and direct stimulus (I_{refl}/I_{stim}) as a function of d for horizontally and vertically polarized light. (C) Example for a RAW image of the choice-box interior in the case of unpolarized stimulus. The sum of the pixel values outside and inside the red rectangle were used to calculate the I_{refl}/I_{stim} ratio.

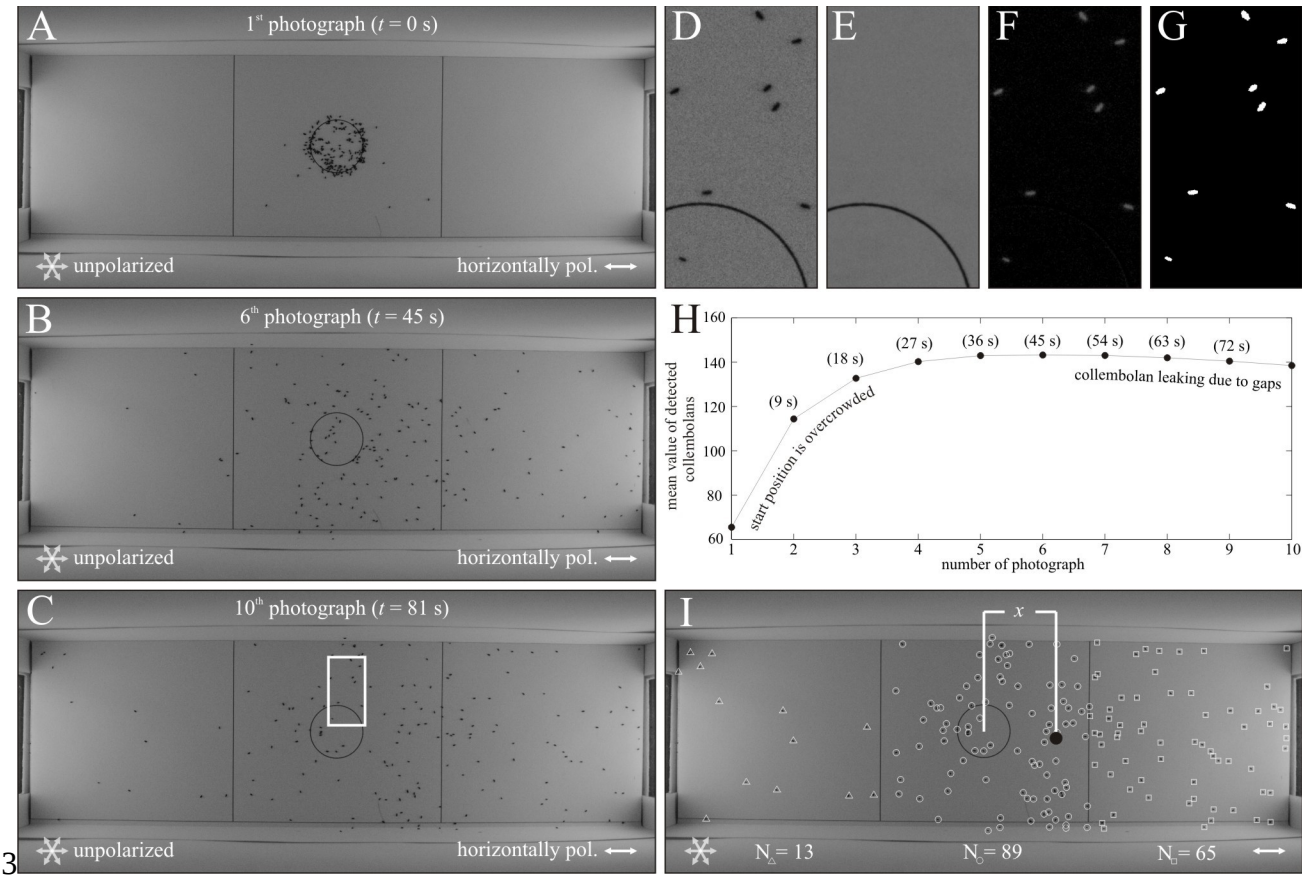


Figure 5: Demonstration of the evaluation process in the case of a trial where the left stimulus was unpolarized and the right was 100% polarized. (A-C) 1st (t = 0 s), 6th (t = 45 s) and 10th (t = 81 s) photograph of the trial. (D) Original 10th photograph (sub-image of C). (E) Median of the 10 photographs taken during the trial. (F) The inverse of E subtracted from the inverse of D. (G) Thresholded image of F. (H) Number of detected springtails as a function of the photograph number taken during the 81 s long test averaged for all 164 trials. The elapsed seconds are also shown in brackets. (I) The result of detection: Triangles, circles and squares show the detected springtails in the left, middle and right third of the choice-box, respectively. The black-filled circle shows the centroid of all detected springtails and x is the shift of the centroid of springtail positions in pixels.

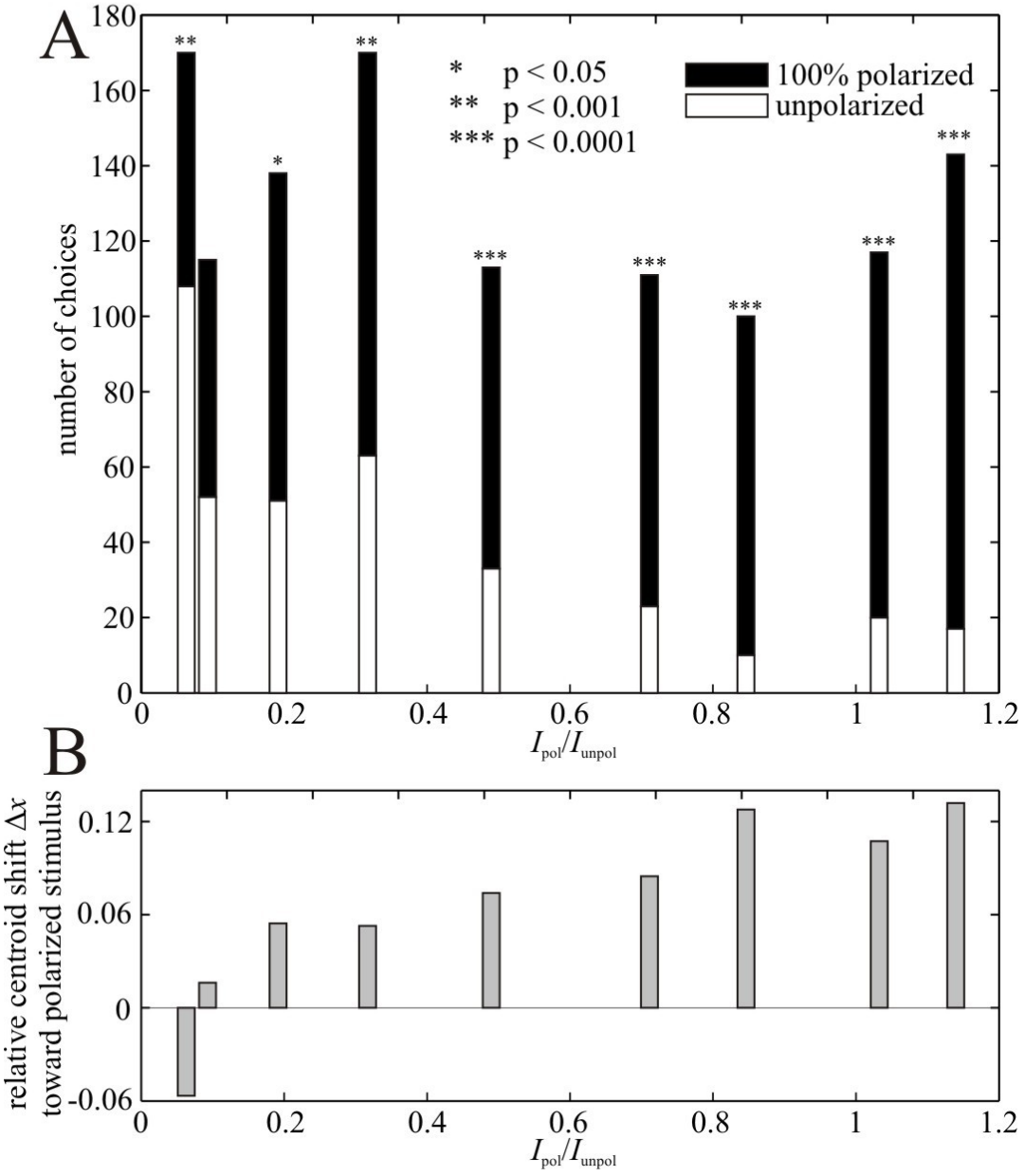


Figure 6: Responses of springtails as a function of the intensity ratio I_{pol}/I_{unpol} of the polarized and unpolarized stimulus in experiment 3. (A) Number of choices at the terminal thirds of the choice-box corresponding to the 100% polarized (black bars) and unpolarized (white bars) stimuli. The asterisks show the significance of the χ^2 tests. (B) Relative centroid shift Δx of springtail positions toward the 100% horizontally polarized stimulus

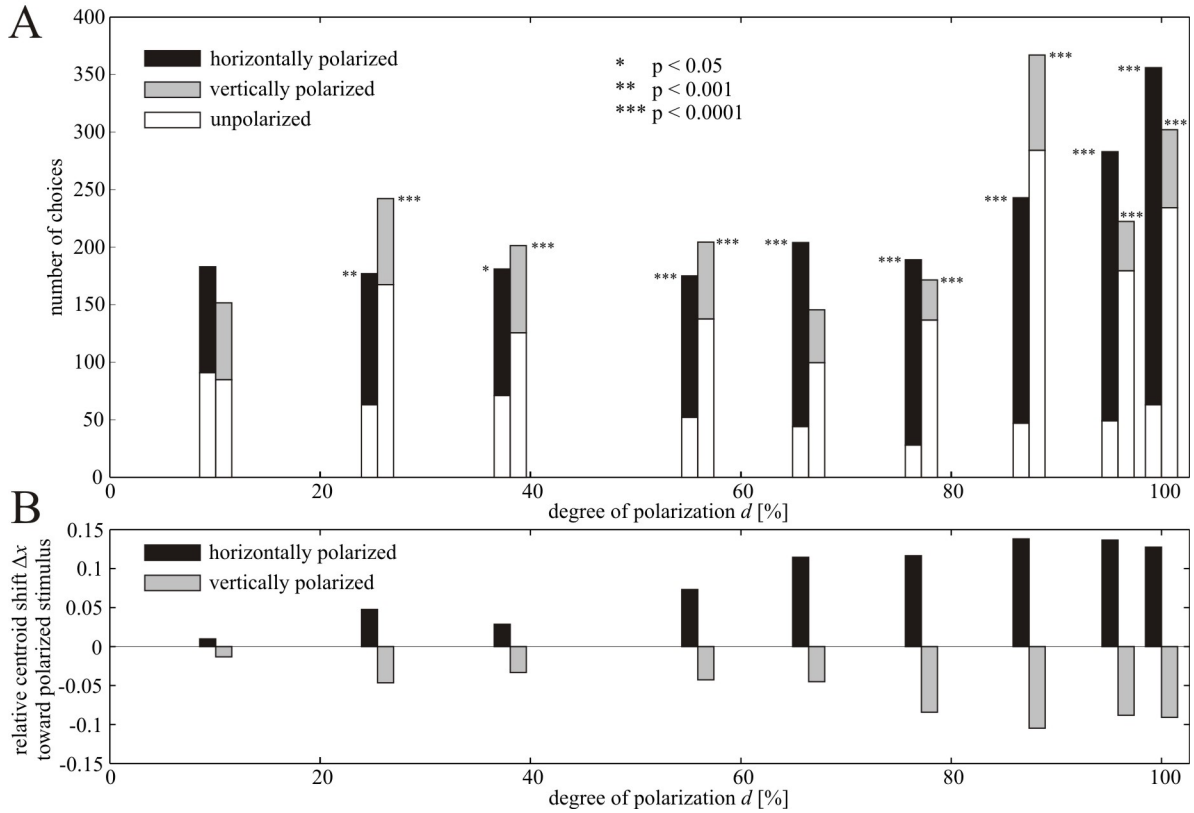
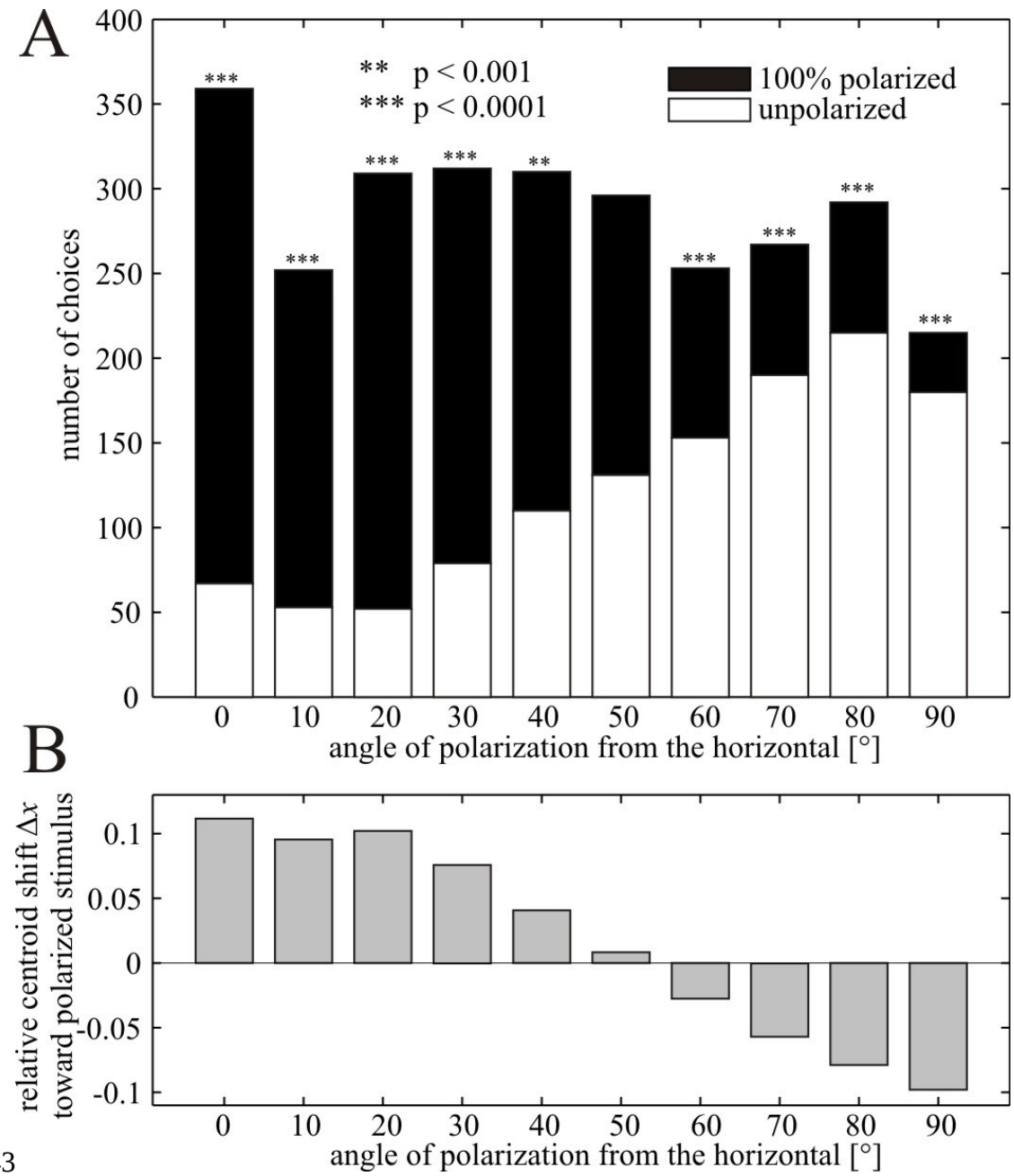


Figure 7: Responses of springtails to horizontally and vertically polarized light in experiment 4 as a function of the degree of polarization *d* against unpolarized stimulus. (A) Number of choices at the terminal thirds of the choice-box corresponding to the polarized (black bars: horizontal, grey bars: vertical) and unpolarized (white bars) stimuli. The asterisks show the significance of the χ^2 tests performed with the modified expected values based on the slight intensity differences between the stimuli. (B) Relative centroid shift Δx of springtail positions toward the polarized stimulus. Black and grey bars correspond to the horizontally and vertically polarized case, respectively.



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646**Figure 8: Responses of springtails in experiment 5 as a function of the angle of polarization α**
647**(from the horizontal).** (A) Number of choices at the terminal thirds corresponding to the 100%
648polarized and unpolarized stimuli. The asterisks show the significance of the χ^2 tests performed with
649the modified expected values based on the slight intensity differences between the stimuli. (B)
650Relative centroid shift Δx of springtail positions toward the 100% polarized stimulus.
651